

Attorney Docket No. P64765US1
Application No. 09/779,461

Amendments to the specification:

At page 3, between lines 2 and 3, insert:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the count number distributions and fits of a 3.8 nM Cy5 solution recorded simultaneously at different time windows T. The weighted residuals for the different time windows are shown in the lower part of the figure.

Figure 2 illustrates the fitting results of simulated data for a mixture of 3 components. The simulated brightness (in kHz) and diffusion time (in μ s) values for the components are: (30 kHz, 192 μ s); (120 kHz, 192 μ s); (120 kHz, 64 μ s). The contributions to the total intensity are 10.8 kHz, 20.4 kHz, and 14.4 kHz, respectively. The graph presents the results of FIMDA from 20 independent realizations of simulations, each corresponding to an experiment of 60 s duration.

Figure 3 illustrates the binding of pTyr-Val-Asn-Val-Lys(Cy5) to SH2. The solid curve results from a hyperbolic fit, yielding a binding constant of $KD = 1.54 \pm 0.14 \mu$ M.

Figure 4 illustrates the experimental setup used in Experiment 2. Radiation emitted by a laser passes an OD filter and reaches a dichroic mirror which reflects the radiation towards an objective having its focus within the sample under study. Fluorescence emitted from the sample passes the objective and reaches the dichroic mirror which is transparent for the fluorescent emission. After passing a bandpass filter and a pinhole, the emission reaches an avalanche photodiode used as part of the detector. By means of a photon counting unit and a computer, specific brightness and diffusion can be determined according to the present invention.

Figure 5 shows 10 count number distributions with time windows 40, 60, 120, 200, 400, 600, 800, 1200, 1600 and 2000 μ s from a 0.8 nM Cy 5 solution.

Figure 6 shows the calculated apparent specific brightness of the dye as a

Attorney Docket No. P64765US1
Application No. 09/779,461

function of counting time interval, evaluated by FIDA.

Reference is now made to Figure 7 which shows one embodiment of an apparatus adapted for use in performing the method according to the present invention. Apparatus 10 comprises a laser 12 which serves as a light source for illuminating the sample by a bundle of coherent monochromatic excitation radiation 14. Excitation radiation 14 is paralleled by a lens 16 and reaches a dichroic mirror 20. Preferably, the angle between the optical axes 18 and the dichroic mirror 20 is 45°. The dichroic mirror 20 reflects the excitation radiation 14 in direction of an objective lens 22 having its focus 24 within a sample volume 26. Sample volume 26 and objective lens 22 are preferably separated from each other by a transparent cover glass 28, e.g. by the bottom of a commercially available microtiter plate which houses the sample. The sample preferably includes fluorescently labeled molecules or other particles. Due to excitation by an appropriate excitation radiation 14, the molecules or other particles present in the sample emit radiation 30. Emission radiation 30 passes the objective lens 22 and reaches the dichroic mirror 20 which is transparent for emission radiation 30. Thereafter, emission radiation passes a filter 34 and a collimator lens 36 on the optical axes 32. A pinhole 38 is situated in the focus of collimator lens 36. Emission radiation 30 passing the pinhole 38 reaches a further lens 40 and, thereafter, is detected by the photodetector 42. Within the pathway of emission radiation 30, in particular between dichroic mirror 20 and photodetector 42, an opaque means 44 is provided through which a central part of the emission radiation 30 cannot pass. This central part of the emission radiation 30 stems from areas on the optical axes 32 in front of or behind the focus 24 of the excitation radiation 14. Only emission radiation 30 that stems from the focus 24 or its direct neighborhood passes the pinhole 38 and reaches photodetector 42. Instead of placing an opaque means 44 within the pathway of emission radiation 30, the pathway of excitation radiation 14 is also suitable for positioning an opaque means

Attorney Docket No. P64765US1
Application No. 09/779,461

44. In particular, an opaque means 44 can be positioned between laser 12 and dichroic mirror 20. The use of an opaque means 44 in the method according to the present invention as described in detail herein improves the signal-to-noise ratio.